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EFFECTS OF GLYPHOSATE ON *VIGNA RADIATA* VAR. ML613 ASSESSED BY MEIOTIC BEHAVIOUR, TOTAL PROTEIN AND GST ACTIVITY

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ABSTRACT

Dose related variation in morphological and yield parameters was observed in Vigna radiata var. ML613 after glyphosate treatment in the first year of sowing. The best performing plant (most vigorous) was selected from the entire population at each dose and its seeds were sown to raise their respective families in the second generation. Post emergence treatment of the same, one lower and one higher dose of glyphosate was given in triplicate to the plants of each family 21 days after sowing. Meiotic studies were done when buds were of appropriate size. Meiotic study of recurrent glyphosate treated Vigna radiata showed micronuclei induction along with chromosomal aberrations. The number of micronuclei decreased with increase in glyphosate concentration while the relative abnormality rate increased with increase in glyphosate concentration. Both these cytological parameters are indicators of mutagenic potential, which was established for glyphosate in our study, and can have long term effect on the qualitative/ nutritive value of the crop plant. Total protein also decreased in the treatments while GST activity increased under the effect of herbicide treatment. A possible interdependence between the oxidative stresses imposed by the herbicide glyphosate in Vigna radiata ML613 has emerged from the above study and the role of antioxidative enzymes like glutathione-S-transferase in ameliorating cytotoxicity is hypothesized.

KEYWORDS: Chromosomal Aberrations, Glutathione-S-Transferase, Micronuclei & Oxidative Stress

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Original Article

INTRODUCTION

The application of herbicides for weed control is an important and integral component of our agricultural system. Though they are used only for weed destruction, a significant residual amount remains in the environment with various manifestations of their effect on different living organisms. It is documented that many of the agrochemicals have cytotoxic and mutagenic properties and are environmentally hazardous (Burnett et al. 1980).

Increased use of pesticides for insect, weed and disease control in the past decade has proved the fact that certain such additives can lead to changes, like inhibition of cell division, induction of chromosomal abnormalities and chromosomal damage (Panneerselvam et al. 2012; Paul et al. 2013; Şutan et al. 2014). Chromosomal aberrations induced by agrochemicals in crop plants are widely used as an indicator of genetic damage. Several scientists have reported cytotoxic effects of agrochemicals on plants (Soriano 1984; Mosuro et al. 1999; Chandra et al. 2002; Saxena et al. 2004; Gul et al. 2006; Mustafa and Arikan 2008; Chaudhari and Chaudhari 2013; Oney and Tabur 2013). The effects of fungicides on mitotic and meiotic behavior in *Allium cepa* as reported by Devi et al. (1991) was induction of chromosomal aberrations. With increasing concentrations of pesticides trifluralin in *Vicia faba* root cells, a decline in mitotic index was observed (Chandra et al. 2002).

Fernandes et al. (2007) reported cellular damage by Trifluralin herbicide in *Allium* cells leading to polyploidy and subsequent generation of micronuclei. Suppression of mitotic activity in the onion root tip cells by parthenin has been reported by Batish et al. (2007). The mutagenic potential of some herbicides using *Vicia faba* as a biological system was tested by Soliman and Ghoneam (2004). Herbicides had the ability to cause mitotic changes varying from reduction in mitotic index, phase distribution to the production of a large number of abnormalities like laggards, bridges, stickiness, C-metaphase as well as micronuclei and multinucleate interphase cells. Micronuclei have been reported to be induced by several herbicides and can be used to assess the mutagenic potential of specific herbicides.

Increased accumulation of antioxidant enzymes in glyphosate treated rice leaf samples suggests that glyphosate treatment generates oxidative stress in plants (Ahsan et al. 2008). Romero et al. (2011) report that glyphosate increased protein and malonialdehyde content, superoxide dismutase and catalase and reduced glutathione levels in *Chlorella kessleri*. Cytotoxic effect on *C. kessleri* was explained to be through a mechanism that involved the induction of oxidative stress. The herbicide TOPIK, as reported by Lukatkin et al. (2012) increased lipid peroxidation (LPO) intensity, superoxide anion ($O_2^{\cdot -}$) generation, total antioxidant activity (AOA), and catalase (CAT) and ascorbate peroxidase (APOX) activity in cereals like wheat, rye and maize, the response being nonlinear and dependent on the herbicide concentration and duration of treatment. As TOPIK concentration increased, LPO and AOA in leaves also increased, confirming the presence of oxidative stress in the cells. Though significant work has been done on the cytotoxicity and physiological consequences of herbicide application, an integrated approach is required for the proper assessment of the effect of the herbicide. The loss in crop yield due to pests and weeds is a major problem in our agriculture. Pesticides are used extensively in agricultural fields to protect pest-induced damage throughout the world. The extensive use of pesticides at high concentrations may induce toxicity problem, which can negatively affect plant growth and development (Yildiztekin et al. 2015).

Review of genotoxicity studies of glyphosate and glyphosate based formulations by Kier and Kirkland (2013) indicated that they did not pose a risk for the production of heritable somatic mutations in humans. However, Kier (2015) reported an increase in the cytokinesis block and micronucleus endpoints which was not consistent with application rates. He also reported increase for blood cell count endpoints at high exposures causing toxicity but suggested no significant genotoxic risk. According to Myers et al. (2016) the use of glyphosate based herbicides has increased about 100 fold since the first decade of its use in the 1970s, making it the world most heavily applied herbicide. Since human exposure to GBH is rising (Myers et al. 2016) and fresh examination of toxicity should be undertaken.

In the present investigation glyphosate tolerant plants of *Vigna radiata* ML613 were selected and their seeds were sown to rise M_2 generation. Each family of tolerant plant was further treated with glyphosate and all treatments were screened for aberrations and number of micronuclei in meiotic preparations, total protein and GST activity.

MATERIAL AND METHODS

Chemicals

The herbicide glyphosate (Round up) [$C_3H_8NO_5P$], is a liquid water-soluble, containing [*N*-(phosphonomethyl) glycine] as its active ingredient (a.i.) was purchased from a local agricultural store in Lucknow, India. All chemicals used in the present study were of analytical grade.

Stock Solutions

Test solution was dissolved in distilled water and desired concentration was made by diluting stock with distilled water.

Herbicide Treatment

Post-emergent treatment of glyphosate of different concentration was given to the 21 days old plants of *Vigna radiata* var. ML613. Best performing plants were selected from each dose and in next generation their respective families were treated again with lower, same and higher concentration of glyphosate. Flower buds of treated plants of M_2 were selected for meiotic studies.

Cytogenetic Assay

For meiotic studies, flower buds were taken from plants of each treatment. Appropriate sized flower buds were fixed in 1:3 iron saturated acetic acid and absolute alcohol for 24h and thereafter transferred to 90% alcohol. Slides were prepared by squashing the anthers in acetocarmine stain, gently heated to fasten the stain and then pressed between the folds of blotting paper. The slides were sealed with wax and studied for meiotic stages, chromosomal behavior and various anomalies; later the slides were made permanent, by some modifications in the acetic-butanol schedule. The cells were then scanned for abnormalities like chromosome clumping, fragmentation, micronuclei formation, etc. The relative abnormality rate (RAR) as calculated using the following formula:

$$\text{Relative Abnormality Rate} = \frac{\text{Total number of abnormal dividing cells}}{\text{Total number of dividing cells}} \times 100$$

Bradford Protein Estimation

Bradford dye-binding assay was used to estimate the protein (Bradford 1976). The Bradford dye consists of Coomassie Brilliant Blue G-250, which binds to arginine, aromatic amino acid and histidine residues of the protein. The absorbance maximum for Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs.

GST Enzyme Assay

The GST activity was determined spectrophotometrically according to Habig et al. (1974), with slight modifications. The assay utilizes CDNB and GSH as substrates. The GST enzyme reaction yields the DNP-GS complex which absorbs at 340 nm. This principle is used to perform the assay. The final *in vitro* assay mixture consisted of 50mM phosphate buffer pH 6.5, 1mM CDNB, 1mM GSH, 0.5mM EDTA and the green leaves extract containing 100 µg proteins. The protein extract was added after incubating the reaction mix at 37ºC for 5 mins. The reaction mixture was made 2.5ml with water and monitoring of the reaction was done at 340nm. The A_{340} at 0 min and 5 min was recorded to calculate ΔOD_{340} and the activity was expressed as µM/mg/min i.e. measure of DNP-GS complex formed.

Statistical Analysis

The data were expressed in percent and the prevalence of significance was determined by analysis of variance (ANOVA) and the significance of mean difference was done by LSD test. ANOVA and LSD test was performed by software IBM SPSS Statistics for Windows, ver. 19.0 (IBM Corp., Armonk, N.Y., USA).

RESULTS

The plants of *Vigna* were treated with glyphosate of different concentration i.e. 0.5mM, 1mM, 2mM, 4mM and 6mM in G_1 and the best performing plants were selected. Their seeds were harvested separately and in next season the seeds of selected plants were sown to raise families which were treated in three ways-

- Plants were given post emergence spray of lower concentration of glyphosate.
- Plants were given post emergence spray of same concentration of glyphosate.
- Plants were given post emergence spray of higher concentration of glyphosate.

(E.g. 1mM tolerant plant was treated with 0.5mM, 1mM and 2mM solutions.)

Cytological Observations

The details of cytological studies – Meiosis I and II are given in Table 1 and 2.

0.5mM Plant: In control plant normal prophase was observed in 25% cells, while spindle disorientation and clumped metaphase I were observed in 7.5% and 5% cells respectively, normal anaphase I in 5% and clumped in 2.5% cells whereas normal telophase I was observed in 5% cells.

In 0.5mM recurrent treated plants, normal anaphase was observed in 17.07% cells, while budding nucleus and clumping aberrations were observed in 1.21, 8.16% cells respectively.

At it's higher i.e. at 1mM; normal prophase, metaphase and anaphase were observed in 39.39, 10.60 and 12.12% cells respectively. Abnormal cells were found only in metaphase I in form of spindle disorientation, clumping and precocious movement in 3.03, 4.54 and 1.51% cells respectively.

RAR increased when the concentration of glyphosate increased i.e. from 65.05 to 80.35% but the percentage of micronuclei decreased from 51.06 to 28.78%.

1mM Plant: In control normal prophase and metaphase I was observed in 33.33% and 11.11% cells respectively. But spindle disorientation and clumped metaphase I were observed in 6.66 and 4.44 cells respectively. Normal anaphase and telophase I were observed in 15.53% and 6.66% cells respectively while clumped metaphase I was observed in 15.55 % cells. In meiosis II only clumped anaphase was observed in 15.55% cells.

In the lower dose treatment i.e. 0.5mM metaphase I was normal (3.65%) and anaphase was clumped in 8.53% cells, while in meiosis II normal anaphase was observed in 54.87% cells, and abnormal cells with spindle disorientation, cytomixis and non synchronous in 18.29, 7.31 and 1.21% respectively, were also observed.

At 1mM recurrent treated plants normal prophase was observed in 16.66% cells and normal metaphase I in 8.33 % cells while abnormal cells with spindle disorientation and clumping were observed in 3.33% and 8.33% cells respectively. Normal anaphase I was observed in 3.33% cells and clumped anaphase I in 15% cells while in meiosis II clumped metaphase and spindle disorientation in 15% cells.

At its higher concentration i.e. at 2mM the normal metaphase was observed in 10.81% cells, budding nucleus was observed in 16.21 % cells of meiosis I. respectively. Clumped metaphase and spindle disorientation were observed in 27.02 and 21.62% cells respectively in meiosis I but in meiosis II only stray anaphase was observed in 2.7% cells.

RAR increased from 35.25 to 89.18% whereas percentage of micronuclei decreased from 2.08% to 0.

2mM Plant- In control normal metaphase II in 7.1%, clumped and spindle disorientation was observed in 3.5% and 17.85 % respectively. Normal anaphase II was seen in 17.85% cells while abnormal cells like bridge, non synchronous, stray in 3.57% cells.

At 1mM dose treatment normal prophase I in 5% and normal metaphase I in 10% cells while clumping in 5% cells were observed. In meiosis II normal metaphase was observed in 5% and abnormal cells like clumping and spindle disorientation was observed in 5% and 2.5% cells respectively.

The 2mM retreated plants showed normal metaphase I in 18.18% cells while only spindle disorientation was found in 2.27% cells in meiosis I. In meiosis II normal telophase was observed in 45.45% cells and abnormal cells were observed in 2.27% cells.

At 4mM treatment normal metaphase and anaphase were observed in 5.70% cells and telophase in 12.85% cells whereas spindle disorientation was observed in metaphase II and anaphase II in 7.14 and 4.28 % cells respectively.

RAR increased with increased conc. of glyphosate i.e. from 75 to 82.85% whereas % of micronuclei decreased from 42.85 to 15.71%.

4mM Plant: At this dose neither lower concentration retreated plants nor higher concentration retreated plants survived. In its control only normal prophase was observed in 27.41% and clumped metaphase I and anaphase I was observed in 1.61% of cells.

RAR increased from control as well as all doses except at 2mM and % of micronuclei increased from all doses.

6mM Plant: At this dose also only control plant survived, and normal prophase, metaphase, anaphase of meiosis II was observed in 7.77% of cells whereas normal telophase I was observed in 3.89% of cells. Clumped metaphase and anaphase were observed in 5.19% of cells but in meiosis II the clumped metaphase was observed in 2.59 % of cells.

RAR increased over the all conc. except at 2mM but the % of micronuclei decreased in comparison to 0.5mM and 4mM.

Table 3 shows the variation in number of micronuclei observed in different stages of meiosis I and II. Higher numbers of micronuclei were observed in meiosis I rather than meiosis II. They outnumbered other aberrations. The percentage of micronuclei in the aberrant cells decreased with increasing concentration of glyphosate. The relative abnormality rate increased with the increasing glyphosate concentration. It was established that glyphosate caused distinct increase in the number of abnormal cells as compared to the control.

Total Protein

Table 4 showed the total protein of *Vigna radiata* ML613 in different herbicide treatments.

0.5mM Plant: Total protein was decreased with increasing concentration of glyphosate (except at 0.5mM) i.e. maximum at 0.5mM (14.09 ± 0.10mg/gm) and minimum at 2mM (8.48 ± 0.01mg/gm) which was significantly ($p<0.01$) decreased in comparison to pure control (12.74 ± 0.25mg/gm), recurrent control (11.84 ± 0.03mg/gm), 0.5mM (14.09 ± 0.10mg/gm) and 1mM (12.51 ± 0.13mg/gm). Maximum and minimum percent decrease over pure control was found in 2.0 mM (33.44%) and 1.0 mM (1.81%).

1mM Plant: Total protein was decreased with increasing concentration of glyphosate except 1mM i.e. maximum at 1mM (15.12 ± 0.56mg/gm) and minimum at 2mM (11.37 ± 1.09mg/gm) which was significantly ($p<0.05$) decreased in comparison to recurrent control (14.87 ± 0.38mg/gm) and 1mM (15.12 ± 0.56mg/gm). In comparison to pure control, in all doses of 1.0 mM, protein content increased except 2.0 mM where it decreased by 10.75%.

2mM Plant: Total protein was decreased with increasing concentration of glyphosate except at 4.0 mM i.e. maximum at recurrent control (14.46 ± 0.20mg/gm) and minimum at 2mM (12.65 ± 0.21mg/gm). In comparison to pure control in all doses of 2.0 mM protein content increased except in 2.0mM where it was decreased up to 0.71%.

4mM Plant: Total protein at recurrent control was observed (13.76 ± 0.08mg/gm) which was increased in comparison to pure control (12.74 ± 0.25mg/gm/8.01%).

6mM Plant: Total protein at recurrent control was observed (6.64 ± 0.13mg/gm) which was significantly ($p<0.01$) decreased in comparison to pure control (12.74 ± 0.25mg/gm) and 4mM recurrent control (13.76 ± 0.08mg/gm). Percent decrease over pure control was 47.67%.

GST Activity

Table 4 showed the GST activity of *Vigna radiata* ML613 in different herbicide treatments.

0.5mM Plant: GST activity was maximum at 1mM (0.10 ± 0.01µm/mg/ml/min) and minimum at recurrent control, 0.5mM and 2mM (0.07 ± 0.00µm/mg/ml/min) which was equal to pure control (0.07 ± 0.00µm/mg/ml/min). Significant difference was observed at 1.0 mM in comparison to both pure control and recurrent control. Percent increase over pure control was also at 1.0mM (42.9%).

1mM Plant: GST activity decreased with increasing concentration of glyphosate except at 0.5 mM i.e. maximum at 0.5mM (0.17 ± 0.02µm/mg/ml/min) and minimum at 2mM (0.10 ± 0.02µm/mg/ml/min). In comparison to pure control (0.07 ± 0.00µm/mg/ml/min) GST activity was significantly ($p<0.01$) increased in all doses. Percent increase over control was found maximum in 0.5mM (142.9%) and minimum in 2.0mM (42.9%).

2mM Plant: GST activity significantly increased with increasing concentration of glyphosate except at 1mM i.e. maximum at 4mM (0.14 ± 0.01µm/mg/ml/min) and minimum at 1mM (0.07 ± 0.00µm/mg/ml/min). Maximum percent increase over pure control was in 4mM (100.0%) and minimum in 1.0mM (0.00%).

4mM Plant: GST activity was observed at recurrent control (0.14 ± 0.01µm/mg/ml/min) which was just double of pure control (0.07 ± 0.00µm/mg/ml/min).

6mM Plant: GST activity was observed at recurrent control (0.07 ± 0.00µm/mg/ml/min) which was just equal to pure control (0.07 ± 0.00µm/mg/ml/min).

DISCUSSIONS

Genotoxicity and mutagenic potential of herbicides has been reported by several workers (Sharma and Vig 2012; Karaismailoglu et al. 2013; Singh and Srivastava 2014; Kumar and Srivastava 2015). Herbicides can cause oxidative stress and active oxygen forms (AOFs) emerging under oxidative stress are known to effect the cytoskeleton structure (Egorova et al. 2001). This disturbs intracellular transport and oxygen consumption carrying intracellular hyperoxia and higher AOFs. Disorders in the stability of cytoskeleton proteins cause inhibition of mitotic events. Takahashi et al. (2008)

have reported mitotic disruption and polynucleated cells by a pyrmidine herbicide NS- 245852 in oat.

In our investigation, glyphosate treatment induced different type of aberrations in PMCs of *V. radiata*. The most frequent aberrations were fragmentation, clumping, precocious movement, cytomixis, unequal separation, bridge, fragmentation and spindle disorientation etc. and the percentage of abnormality containing cells was different at each dose of glyphosate. Similarly Singh and Srivastava (2014) investigated genotoxic effect of herbicides pendimethalin and glyphosate in *V. mungo* and found various chromosomal aberrations like disturbed polarity, spindle disturbance, anaphase bridges and laggards. They also observed large number of cells with micronuclei as we observed in our study also. The occurrence of micronuclei has been regarded as reliable parameter for the genotoxicity, clastogeneticity/mutagenecity of an agent (Patel et al. 2009). The variation in number of micronuclei observed in different stages of meiosis I and II. Higher numbers of micronuclei were observed in meiosis I rather than meiosis II. They outnumbered other aberrations. The percentage of micronuclei in the aberrant cells decreased with increasing concentration of glyphosate. The relative abnormality rate increased with the increasing glyphosate concentration. It was established that glyphosate caused distinct increase in the number of abnormal cells as compared to the control.

Cytogenetic effects of avenoxan on meiotic chromosomes of *Allium cepa* and pollen sterility have been reported. The type of the abnormalities induced were chromosome stickiness, bridges, laggards, univalent's, quadrivalents and micronuclei (Kaymak and Muranli 2005). Meiotic chromosome aberrations were observed in pollen mother cells (PMC's) of *Secale cereale* treated with glyphosate and ethephon, the most frequent aberrations found in plants were clumping of bivalents and unsynchronized division at the heavier dose level; laggards, fragments and micronuclei in low frequency (Boyle and Evans 1974).The herbicide terbutryn induced upto 11.3% chromosomal abnormalities in cells undergoing meiosis in *Vicia faba* (Badr et al. 1987). Several herbicides have been known to cause chromosomal abnormalities. Soriano (1984) observed herbicide-induced chromosomal aberrations in sorghum. Unrau and Larter (1952) studied the effect of 2,4-D on meiosis in cereals. Liang et al. (1969) observed a preponderance of aneuploidy and polyploidy in grain sorghum after atrazine and 2,4-D treatments. The abnormalities induced were chromosome stickiness, bridges, laggards, univalent's, quadrivalents and micronuclei.

Nutritive composition of seeds was also affected by the pesticide. Our study revealed that the glyphosate treatment decreased the protein content of the crop. Similarly El Tayeb and Zaki (2009) also reported that roundup caused significant decrease in soluble proteins in all concentrations and durations in stem, leaves and roots of *Vicia faba*. Decrease in grain protein by treatment of isoproturon and atrazine was observed in green gram (Khan et al. 2006). Rajashekar et al. (2012) also found that pendimethalin treatment in maize seedlings reduced soluble protein content. Metosulum treatment in *Vicia faba* decreased soluble proteins and free amino acids content in all organs in comparison with those of control plants after 12 and 24h but proline content was increased (Badr et al. 2013). Abo-El-Seoud and Frost (1998) sprayed dimethoate and pirimicarb at the recommended dose on growing plants of wheat and noticed a decrease in chlorophyll, sugars and carbohydrates, total proteins and RNA content of wheat shoots as a function of the applied pesticides. Kumar (2012) found that herbicides 2, 4-Dichlorophenoxy acetic acid and Isoproturon reduce the carbohydrate and protein content gradually from lower to higher concentration of herbicides.

GST activity is known to be induced by diverse herbicides and safeners in several plant species (Andrews et al. 2005). There are several reports which have clearly demonstrated an increase in GST activity after treatment with herbicides like terbuthylazine, metolachlor, fluorodifen and fenoxaprop-ethyl (Edwards and Cole 1996).

The conjugation of herbicides with GSH catalyzed by GSTs is also known. Cataneo et al. (2003) showed an increase in GST activity in maize shoots after glyphosate treatment. Glyphosate was applied at concentrations of 1,000, 2,500 and 5,000 ppm at four developmental stages (9, 16, 23 and 30 days after emergence). The study proposed that GSTs might be involved in the degradation of glyphosate in maize plants. The GST activity showed an increase in the glyphosate treated leaves of *Vigna radiata*. The increase in GST activity is in response to the ROS generated by herbicide treatment. Basantani et al. (2011) have reported the elevated expression of antioxidant enzymes and induction of tau class GST after glyphosate treatment in *Vigna radiata*. Jain and Bhalla-Sarin (2001) observed differences in the sensitivity of three groundnut cultivars (JL24, CO2, and TMV2) to glyphosate. They reported an increase in the specific activity of GST at sub-lethal herbicide concentration (0.3mM), and maximum induction was seen between 9 and 12 days of the treatment. Glyphosate tolerant cell lines of groundnut (JL24) selected *in vitro* showed an elevated basal level of GST activity. Sergiev et al. (2006) reported a considerable gradual increase in GST activity in maize plants after glyphosate applications.

CONCLUSIONS

Meiosis is an event of high evolutionary stability which culminates in a reduction of chromosome number. Gamete viability depends on a normal and uneventful reduction division. The cytological events leading to gamete formation are governed by a large number of genes that act from pre-meiotic to post-meiotic mitosis. According to Datta et al. (2011) antioxidant metabolism has been shown to be important in determining the ability of plants to survive in oxidative stress, an upregulation of these enzymes would help to reduce the built up ROS. The level of free radical formation was high immediately after gamma irradiation, which was reflected by the high percentage of chromosomal abnormalities. A possible interdependence between the oxidative stresses imposed by the herbicide glyphosate in *Vigna radiata* ML613 has emerged from the above study and the role of antioxidative enzymes like glutathione-S- transferase in ameliorating cytotoxicity is hypothesized.

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REFERENCES

1. *Abo-El-Seoud, M. A and Frost, M. 1998. Biochemical changes in wheat plants as affected by residues of dimethoate and pirimicarb. Environmental Management and Health, 9:188–193.*
2. *Ahsan, N., Dong-Gi, L., Ki-Won, L., Alam, I., Sang-Hoon, L., Jeong, D. B. and Byung-Hyun, L. 2008. Glyphosate-induced oxidative stress in rice leaves revealed by proteomic approach. Plant Physiology and Biochemistry, 46:1062–1070.*
3. *Andrews, C. J., Cummins, I., Skipsey, M., Grundy, N. M., Jepson, I., Townson, J. and Edwads, R. 2005. Puriication and characterization of a family of glutathione transferases with roles in herbicide detoxification in soybean (Glycine max L.); selective enhancement by herbicides and herbicide safeners. Pesticide Biochemistry and Physiology, 82: 205–219.*
4. *Badr, A., Hamoud, M. A. and Haroun, S. A. 1987. Effect of the herbicide terbutryn on meiosis, yield and mitotic chromosomes in C_2 plants of Vicia faba L. Biologia Plantarum, 29:70–72.*
5. *Badr, A., Zaki, H., Germoush, M. O., Tawfeek, A. Q. and El-Tayeb, M. A. 2013. Cytophysiological impacts of Metosulam herbicide on Vicia faba plants. Acta Physiologiae Plantarum, 35:1933–1941.*
6. *Basantani, M., Srivastava, A. and Sen, S. 2011. Elevated antioxidant response and induction of tau class glutathione S transferase after glyphosate treatment in Vigna radiata (l.) Wilczek. Pestic Biochem Physiol, 99:111–117.*

7. *Batish, D. R., Singh, H. P., Kohli, R. K., Kaur, S., Saxena, D. B. and Yadav, S. 2007. Assessment of phytotoxicity of parthenin. Z Naturforsch, 62:367–372.*

8. *Boyle, W. S. and Evans, J. O. 1974. Effects of glyphosate and ethephon on meiotic chromosomes of Secale cereale L. Journal of Heredity, 65:250.*

9. *Bradford, M. M. 1976. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemistry, 72:248–254.*

10. *Burnett, C., Jacobs, M., Seppala, A. and Shubik, P. 1980. Evaluation of toxicity and carcino geneticity of hair dyes. Journal of Toxicology and Environmental Health, 6:247.*

11. *Cataneo, A. C., Destro, G. F., Ferreira, L. C., Chamma, K. L. and Sousa, D. C. 2003. Glutathione S transferase activity on the degradation of the herbicide glyphosate in maize (Zea mays) plants. Planta Daninha, 21:307–312.*

12. *Chandra, B. R., Saharan, R. P. and Sareen P K (2002) Clastogenic effect of trifluralin in Vicia faba. Journal of Cytology and Genetics, 3:201–203.*

13. *Chaudhari, S. V. and Chaudhari, P. S. 2013. Effect of pesticides and fertilizers on development of root of onion Allium cepa (Linn.). International Journal of Innovations and Research Development, 2:1075–1081.*

14. *Datta, S. K., Kumar, S., Chakrabarty, D., Verma, A. K. and Banerjee, B. K. 2011. Gamma ray induced chromosomal aberrations and enzyme related defense mechanism in Allium cepa L. Caryologia, 64:388–390.*

15. *Devi, P., Kiranmai, V. and Padmavathi, T. 1991. Pesticide induced cytological abnormalities in Allium cepa L. Journal of Cytology and Genetics, 26:13–18.*

16. *Edwards, R., and Cole, D. J. 1996. Glutathione transferases in wheat (Triticum) species with activity towards fenoxaprop-ethyl and other herbicides. Pesticide Biochemistry and Physiology, 54:96–104.*

17. *Egorova, A. B., Uspenskaya, Y. A., Mikhutkina, S. V. and Stavitskaya, E. Y. 2001. Damage of cytoskeleton and cell membranes in apoptosis. Adv Cur Biol, 121:502–510.*

18. *El-Tayeb, M. A. and Zaki, H. 2009. Cytophysiological reponses of Vicia faba to a glyphosate based herbicide. American-Eurasian Journal of Agronomy, 2:168–175.*

19. *Fernandes, T. C. C., Mazzeo, D. E. C. and Marin-Morales, M. A. 2007. Mechanism of micronuclei formation in polyploidizated cells of Allium cepa exposed to trifluralin herbicide. Pesticide Biochemistry and Physiology, 88:252–259.*

20. *Gul, T., Kaymark, F. and Murnali, F. D. G. 2006. Genotoxic effects of avenoxan on Allium cepa L. and Allium sativum L. Caryologia, 59:241–247.*

21. *Habig, W. H., Pabst, M. H. and Jakoby, W. B. 1974. Glutathione S transferases: the first enzymatic step in mecapturic acid production. Journal of Biological Chemistry, 249:7130–7139.*

22. *Jain, M. and Bhalla-Sarin, N. 2001. Glyphosate induced increase in glutathione S transferase activity and glutathione content in groundnut (n L.). Pesticide Biochemistry and Physiology, 69:143–152.*

23. *Karaismailoglu, M. C., Inceer, H. and Hayirliglu-Ayaz, S. 2013. Effects of Quizalofop-p-Ethyl herbicide on the somatic chromosomes of Helianthus annuus (Sunflower). Ekoloji, 22:49–56.*

24. *Kaymak, F. and Muranli, D. G. 2005. The cytogenetic effects of avenoxan on Allium cepa and its relation with pollen sterility. Acta Biologica Hungarica, 56:313–321.*

25. *Khan, M. S., Chaudhry, P., Wani, P. A. and Zaidi, A. 2006. Biotoxic effects of the herbicides on growth, seed yield and grain protein of greengram. Journal of Applied Sciences and Environmental Management, 10:141–146.*

26. *Kier, L. D. 2015. Review of genotoxicity biomonitoring studies of glyphosate-based formulations. Critical Reviews in Toxicology, 45(3):209–218.*

27. *Kier, L. D. and Kirkland, D. J. 2013 Review of genotoxicity studies of glyphosate and glyphosate based formulations. Critical Reviews in Toxicology, 43:283–315.*

28. *Kumar, G. and Srivastava, A. 2015. Comparative genotoxicity of herbicide ingredients glyphosate and atrazine on root meristem of buckwheat (Fagopyrum esculentum Moench). Jordan Journal of Biological Sciences, 8:221–226.*

29. *Kumar, S. 2012. Effect of herbicides on carbohydrate, protein and electrophoretic protein bands content in Triticum aestivum. International Journal of Food Agriculture and Veterinary Sciences, 2:13–25.*

30. *Liang, G. H. L., Feltner, K. C. and Russ, O. G. 1969. Meiotic and morphological response of grain Sorghum to Atrazine, 2,4-D, Oil and their combinations. Weed Science, 17:8–12.*

31. *Lukatkin, A. S., Gar'kova, A. N., Bochkarjova, A. S., Nushtaeva, O. V. and Teixeira da Silva, J. A. 2012. Treatment with the herbicide TOPIK induces oxidative stress in cereal leaves. Pesticide Biochemistry and Physiology, 105:44–49.*

32. *Mosuro, A. A., Bakare, A. A., Koofreh, M. A. and Ngenwi, A. A. 1999. Genetic safety evaluation of pesticides using the Allium cepa assay. Journal of Cytology and Genetics, 34:173–182.*

33. *Mustafa, Y. and Arikan, E. S. 2008. Genotoxicity testing of Quizalofop-p-ethyl herbicide using the Allium cepa anaphasetelophase chromosome aberration assay. Caryologia, 61:45–52.*

34. *Myers, J. P., Antoniou, M. N., Blueberg, B., Carroll, L., Colborn, T., Everett, L. G., Hansen, M., Landrigan, P. J., Lanphear, B. P., Mesnage, R., Vandenberg, L. N., Saal, F. S. V., Welshons, W. V., Benbrook, C. M. 2016. Concerns over use of glyphosate-based herbicides and risks associated with exposures: a concern statement. Enviornmental health, 15:1-13.*

35. *Oney, S. and Tabur, S. 2013. Cytogenetical and molecular responses of exogenous potassium sulphate for tolerance to extreme temperatures in Vicia faba L. Journal of Pure Applied Microbiology, 7:663–670.*

36. *Panneerselvam, N., Palanikumar, L. and Gopinathan, S. 2012. Chromosomal aberrations induced by Glycidol in Allium cepa L. root meristem cells. International Journal of Pharmaceutical Sciences and Research, 3:300–304.*

37. *Patel, P. B., Trivedi, P. J., Brahmbhatt, M. M., Shukla, S. N., Shah. and Bakshi, S. R. 2009 Micronuclei and chromosomal aberrations in healthy tobacco chewers and controls: A study from Gujarat, India. Archives of Oncology, 17:7–10.*

38. *Paul, A., Nag, S. and Sinha, K. 2013. Cytological effects of Blitox on root mitosis of Allium cepa L. International Journal of Scientific Research Publication, 3:1–7.*

39. *Rajashekar, N. Prakasha. And Murthy, T. C. S. 2012. Seed germination and physiological behavior of maize (cv. Nac-6002) seedlings under abiotic stress (Pendimethalin) condition. Asian Journal of Crop Science, 4:80–85.*

40. *Romero, D. M., Ríos de Molina, M. C. and Juárez, A. B. 2011. Oxidative stress induced by a commercial glyphosate formulation in a tolerant strain of Chlorella kessleri. Ecotoxicology and Environmental Safety, 74:741–747.*

41. *Saxena, P. N., Chauhan, L. K. S., Chandra, S. and Gupta, S. K. 2004. Genotoxic effects of Diuron contaminated soilon the root meristem cells of Allium sativum: A possible mechanism of chromosome damage. Toxicology Mechanisms and Methods, 14:281–286.*

42. *Sergiev, I. G., Alexieva, V. S., Ivanov, S. V., Moskova II and Karanov, E. N. 2006. The phenylurea cytokinin 4PU-30 protects maize plants against glyphosate action. Pesticide Biochemistry and Physiology, 85:139–146.*

43. *Sharma, S. and Vig, A. P. 2012. Genotoxicity of Atrazine, Avenoxan, Diuron, Quizalofop-P-ethyl herbicides using the Allium cepa root chromosomal aberration assay. Terrestrial and Aquatic Environmental Toxicology, 6: 90–95.*

44. *Singh, N. and Srivastava, A. 2014. Biomonitoring of genotoxic effect of glyphosate and pendimethalin in Vigna mungo populations. Cytologia, 79: 173–180.*

45. *Soliman, M. I., Ghoneam, G. T. 2004. The mutagenic potential of some herbicides using Vicia faba as a biological system. Biotechnology, 3:140–154.*

46. *Soriano, J. D. 1984. Herbicides induced chromosomal aberrations and inheritance of digenic seedling mutation in Sorghum, x. Cytologia, 49:201–207.*

47. *Şutan, N. A., Popescu, A., Mihaescu, C., Soare, L. C. and Marinescu, M. V. 2014. Evaluation of cytotoxic and genotoxic potential of the fungicide ridomil in Allium cepa L. Analele Stiint. Univ. Al. I. Cuza Iasi, Sect. II a. Biol. Veget, 60:5–12.*

48. *Takahashi, S., Yamada, H., Yamada, T. and Kawana. 2008. Mitotic disruption by a novel pyrimidine herbicide, NS- 245852, in oat (Avena sativa L.) roots. Weed Biology and Management 1:182–188.*

49. *Unrau, J., Larter, T. N. 1952. Cytological responses of cereals to 2,4-D. I. A study of meiosis of plants treated at various stages of growth. Canadian Journal of Botany, 30:22–27.*

50. *Yildiztekin, M., Kaya, C., Tuna, A. L. and Ashraf, M. 2015. Oxidative stress and antioxidative mechanisms in tomato (Solanum lycopersicum L.) plants sprayed with different pesticides. Pakistan Journal of Botany, 42(2):717–721.*